

Total RNA isolation from *A. gossypii*

Total DNA/RNA extraction

- This protocol is described for a liquid culture (10^8 spores incubated for 2 days at 37°C).
- Harvest mycelium by filtration, wash once with DEPC treated ddH₂O
- Use pellet immediately for extraction, alternatively freeze pellet in liquid nitrogen and store at -80°C
- Resuspend pellet in 5ml phenol (Roth), 5ml breakage buffer (75mM ammonium acetate, 10mM EDTA), 500 µl 10% SDS, and 1g of glass beads (0.25-0.50 mm, Roth).
- Vortex vigorously for 5 min
- Incubate solution at 65°C for 15min (Vortex the mix every 30s)
- Transfer aqueous phase into fresh tube
- Add 5ml phenol/Chloroform (1:1)
- Vortex vigorously
- Centrifuge 10 min at 10000 rpm
- Transfer aqueous phase into fresh tube
- Add 5ml Chloroform
- Vortex vigorously
- Centrifuge 5 min at 10000 rpm
- Transfer aqueous phase into fresh tube
- Precipitate by adding 750 ammonium acetate and 2.5x volumes of ice cold ethanol
- Leave on ice for 10 min
- Centrifuge 10 min at 10000 rpm
- Wash once with 70% ethanol
- Dry pellet at room temperature for several minutes

DNase treatment

- Add 10µl DNase buffer pre-chilled on ice
- Add 2µl DNase I (Amersham) and mix
- Spin for a few seconds
- Incubate at 37°C for 30 min
- Add 10µl 3M Na-acetate pH5.2 and gently mix by pipeting and down

RNA extraction

- Add 100µ phenol
- Vortex vigorously
- Centrifuge 1 min at 13000 rpm (Room temperature)
- Transfer aqueous phase into fresh tube
- Add 200µl Phenol/Chloroform (1:1)
- Vortex vigorously
- Centrifuge 1 min at 13000 rpm
- Repeat extraction twice with 200µl Chloroform
- Transfer aqueous phase into fresh tube
- Precipitate by adding 500µl of ice cold ethanol
- Incubate overnight at -20°C

- Centrifuge 60 min at 14000 rpm
- Wash once with 500µl 70% ethanol
- Dry pellet at room temperature for several minutes
- Resuspend pellet in 50-100µl RNase free water
- Incubate on ice for 10 min
- Incubate at 60°C for 10 min

The RNA preparation can be kept for several weeks at -70°C

DNase buffer

40mM Tris-Cl pH7.5

6mM MgCl₂